UNIVERSITI PUTRA MALAYSIA

ISOLATION AND IDENTIFICATION OF ANTIMICROBIAL AND CYTOTOXIC COMPOUNDS FROM GARCINIA CANTLTYNA AND G. NIGROLINEATA

KHALID AHMAD SHAKER SHADID.

ISOLATION AND IDENTIFICATION OF ANTIMICROBIAL AND CYTOTOXIC COMPOUNDS FROM *GARCINIA CANTLEYNA* AND *G. NIGROLINEATA*

KHALID AHMAD SHAKER SHADID

DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA

2005

ISOLATION AND IDENTIFICATION OF ANTIMICROBIAL AND CYTOTOXIC COMPOUNDS FROM *GARCINIA CANTLEYNA* AND *G. NIGROLINEATA*

By

KHALID AHMAD SHAKER SHADID

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

September 2005

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

ISOLATION AND IDENTIFICATION OF ANTIMICROBIAL AND CYTOTOXIC COMPOUNDS FROM *GARCINIA CANTLEYNA* AND *G. NIGROLINEATA*

By

KHALID AHMAD SHAKER SHADID

September 2005

Chairman: Professor Md. Nordin Hj. Lajis, PhD

Institute: Bioscience

Eleven species of *Garcinia* (Guttiferae) from the flora of Malaysia were screened *in vitro* for antimicrobial and cytotoxic activities. Disc diffusion and MTT methods were utilized to screen the antimicrobial and cytotoxic effects, respectively. On the basis of the screening results and literature review of the tested plants, *Garcinia cantleyana* and *Garcinia nigrolineata* were selected for phytochemical investigations.

The investigations of the chloroform extract of *Garcinia cantleyana* by a combination of different chromatographic techniques led to the isolation of eight new natural products: three caged tetraprenylated xanthonoids; cantlyanone A, cantlyanone B and cantlyanone C, four caged triprenylated xanthonoids; cantlyanone D, cantlyanone E, cantlyanone F and cantlyanone G, and 1,4,6,8-tetrahydroxy-5-(2-methylbut-3-en-2-yl)-9*H*-xanthen-9-one (cantleyanaxanthone). Six known compounds namely, glutin-5-en-3β-ol, a mixture of stigmasterol and

β-sitosterol, guadichaudion H, garbogiol and for the first time in *Garcinia* species the isolation of sesquineolignan (Macranthol).

All caged-polyprenylated xanthonoids were found to exhibit significant cytotoxicity against several cancer cell lines with IC_{50} values from 0.2-3 µM. Broth microdilution method was used to determine antibacterial activity for the isolated compounds; the results showed strong antibacterial activity against *staphylococcus aureus* ATCC 335591 for Cantleyanone F with MIC value of 31.25 µg/ml.

Sesquineolignan (Macranthol) which was isolated for the first time in this genus showed cytotoxic IC_{50} values of 4.17, 3.70, 1.53, 2.53 µg/ml against MDA-MB-231, MCF-7, CaOV-3 and HeLa, respectively, and antibacterial activity with an MIC value of 3.91 µg/ml activity against *staphylococcus aureus* ATCC 335591, the result of which is remarkable.

From the methanolic extract of *Garcinia nigrolineata* leaves, three compounds were isolated, namely a mixture of stigmasterol and β-sitosterol, friedelin, and for the first time methyl putranjivate from *Garcinia nigrolineata*. Bioassays was carried out, but these compounds were inactive against several cell lines.

The structures of all compounds were carried out with the help of chemical and modern spectroscopic techniques (UV, IR, MS, ^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H-^{1}H COSY, HMQC, and HMBC).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk ijazah Doktor Falsafah

PEMENCILAN DAN PENGENALPASTIAN SEBATIAN-SEBATIAN SITOTOKSIK DAN ANTIMIKROBIAL DARIPADA *GARCINIA CANTLEYNA* DAN *G. NIGROLINEATA*

Oleh

KHALID AHMAD SHAKER SHADID

September 2005

Pengerusi: Profesor Md. Nordin Hj. Lajis, PhD

Institut: Biosains

Sebelas spesies *Garcinia* daripada Malaysia telah dikaji secara *in vitro* untuk menentukan aktiviti-aktiviti antimicrobial dan sitotoksik. Kaedah pembauran cakera dan MTT telah diguna untuk menentukan kesan antimicrobial dan sitotoksik. *G. cantleyana dan G. nigrolineata* telah dipilih untuk kajian lebih lanjut berdasarkan keputusan aktiviti-aktiviti biologi dan kajian terdahulu.

Kajian terhadap ekstrak kloroform *G. cantleyana* dengan menggunakan kombinasi berbagai teknik kromatografi yang berbeza telah berjaya memencilkan lapan sebatian semulajadi yang baru: tiga sangkar tetraprenil xanthonoid; kantlianon A, kantlianon B, kantlianon C, empat sangkar triprenil xanthonoid; kantlianon D, kantlianon E, kantlianon F, dan kantlianon G serta kantlianaxanthon. Enam sebatian yang telah diketahui yaitu glutin-5-ena-3β-ol, campuran stigmasterol dan β-sitosterol, guadicaudion H, garbogiol, dan buat pertama kali dalam spesies *Garcinia* pemencilan seskuineolignan.

Kesemua sangkar poliprenil xanthonoid telah menunjukkan aktiviti sitotoksik yang signifikan terhadap beberapa talian sel dengan nilai 50% perencatan dari 0.2-3 μM. Kaedah "broth microdilution" telah digunakan untuk menentukan aktiviti antibakteria kesemua sebatian yang telah dipencilkan. Katlianon F telah menunjukkan aktiviti antibakteria yang tinggi dengan nilai MIC 31.25 μg/ml.

Seskuineolignan telah dipencilkan buat kali pertama dalam genus ini telah menunjukkan aktiviti sitotoksik dengan nilai 50% perencatan 4.17, 3.70, 1.53, 2.53 μg/ml terhadap sel MDA-MB-231, MCF7, CaOV-3, dan HeLa, setiap satu. Sebatian ini juga menunjukkan aktiviti antibakteria terhadap *staphylococcus aureus* ATCC 335591, dengan nilai MIC 3.91 μg/ml.

Tiga sebatian berjaya dipencilkan daripada ekstrak methanol daun *G. nigrolineata* iaitu campuran stigmasterol dan β-sitosterol, fridelin, dan metil putranjivat yang buat pertama kali dipencilkan daripada *G. nigrolineata*. Sebatian ini didapati tidak aktif terhadap semua talian sel yang diuji.

Struktur kesemua sebatian telah ditentukan dengan menggunakan teknik kimia dan spektroskopik moden (UV, IR, MS, ^{1}HNMR, ^{13}NMR, DEPT, ^{1}H-^{1}H COSY, HMQC, and HMBC).

ACKNOWLEDGEMENTS

All praise to **Allah** most Gracious, Most Merciful, Who, Alone brings forgiveness and light and new life to those who call upon Him; and to Him is the dedication of this thesis.

"Read! In the Name of your Lord Who has created (all that exist).
He has created man from a clot.
Read! And your Lord as the Most Generous.
Who has taught (the writing) by the pen.
He has taught man that which he knew not."
Qur'an 96: 1-5

We praise Allah for His great loving kindness, which has brought us all together to tell and encourage each other and mankind with stories of His care, and leading. In so doing, I also thank to those who answered His call, who have started their journey upon the Straight Path of Allah. All respect for our Holy Prophet (Peace be upon him), who guided us to identify our creator

I am whole-heartedly thankful to my research supervisor, Prof. Dr. Md. Nordin Haji Lajis, Head of the Laboratory of Natural Products, institute of Biosience for his encouragement, exceptional ideas, and tireless optimism that have kept me going, and for providing the excellent facilities for my work.

I am thankful to Prof. Dr. Abdul Manaf, who kindly guided me into the 'Wonderland of Animal Cell Cultures' and for being positive and encouraging about my research.

My thanks are extended to Associate Professor Dr. Daud Ahmad, who has encouraged me to persevere with the biological assays despite the initial undesirable results, and to Prof Dr. Ahmad Sazali, for his keen interest precious attention and guidance.

I am also thankful to Associate Professor Dr Khozirah shaari, for her kindness, valuable suggestions and persistent help in the field of NMR. I have learned so much from her.

Sincere thanks are due to my colleagues at the Laboratory of Natural Products, Drs. Rohaya, Faridah, Koushik, Dharma, and Habsah, Suryati, and Maulidiani who have made the period when I worked on the thesis very inspiring.

In addition, I am grateful to science officers, Din, Zurina, Mazina, Abby and Julia, all of you have been so helpful. Special thanks to Shamsul for getting me samples of plant material. Many thanks to Normah, who helped with bioassays, thank you so much.

Finally, my warmest thanks are due to my to my family – parents Ahmad Shaker Shadid and Nehal Shadid, brother Nader, sisters Neda'a and Sana'a as well as my wife Ihsan Mkhaimar – for their love understanding encouragement and never failing support during all these years.

KHALID AHMAD SHAKER SHADID
June 2005

I certify that an Examination Committee met on 27th September 2005 to conduct the final examination of Khalid Ahmad Shaker Shadid on his Doctor of Philosophy thesis entitled "Isolation and Identification of Antimicrobial and Cytotoxic Compounds from *Garcinia cantleyna* and *G. nigrolineata*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

MAWARDI RAHMANI, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

GWENDOLINE EE CHENG LIN, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

KHOZIRAH SHAARI, PhD
Associate Professor
Institute of Bioscience
Universiti Putra Malaysia
(Internal Examiner)

MUHAMMAD IQBAL CHOUDHARY, PhD
Professor
H. E. J. Research Institute of Chemistry
University of Karachi
Karachi
(External Examiner)

GULAM RUSUL RAHMAT ALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 22 NOV 2005

The thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

MOHD. NORDIN HJ. LAJIS, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

ABDUL MANAF ALI, PhD
Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

DAUD A. ISRAF ALI, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

AHMAD SAZALI, PhD
Professor
Institute of Science
Universiti Teknologi MARA
(Member)

AINI IDERIS, PhD
Professor/ Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 08 DEC 2005

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degree at UPM or other institutions.

KHALID AHMAD SHAKER SHADID

Date: **28 SEP 2005**

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

δ	Chemical shift in ppm
°C	Degree in Celsius
$[\alpha]_D$	Specific rotation at sodium D-line
bp	Boiling point
br	Broad
BuOH	Butanol
^{13}C	Carbon-13
d	Doublet
dd	Doublet of doublet
ddd	Doublet of doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	Dimethylsulfoxide
EtOAc	Ethyl acetate
eV	Electron volt
FTIR	Fourier Transform Infra-Red
GC-MS	Gas Chromatography-Mass Spectrometry
^{1}H	Proton
gHMBC	Gradient Heteronuclear Multiple Bond Correlation
gHSQC	Gradient Heteronuclear Single-Quantum Coherence
gCOSY	Gradient Correlation Spectroscopy
HREIMS	High Resolution Electron Impact Mass Spectrum
EIMS	Electron Impact Mass Spectrum
ESIMS	Electro-Spray Ionization Mass Spectrum
Hz	Hertz
IR	Infrared
J	Coupling in Hz
Lit.	Literature
m	Multiplet
m/z	Mass per charge
MeOH	Methanol
MHz	MegaHertz
m.p.	Melting point